

**IN THE CLAIMS**

1-11 (Cancelled)

12. (Currently amended): A method for preparing a target protein with a carboxy-terminal thioester, comprising:

- (a) expressing in a host cell, a recombinant precursor protein ~~in-a host cell, the precursor protein comprising the target protein fused at its carboxy terminus to an intein, the intein having an amino-terminus and a carboxy-terminus wherein the amino-terminus is fused to the target protein and the carboxy-terminus and is optionally fused to a binding protein binding-domain, the intein being selected from a naturally occurring native intein, an intein derivative or an intein mutant; wherein the intein is capable of being cleaved from the protein in the presence of 2-mercaptopethanesulfonic acid; and~~
- (b) contacting the expressed precursor protein with 2-mercaptopethanesulfonic acid and inducing cleavage of the intein from the precursor protein so as to form the target protein having the carboxy-terminal thioester.

13. (Currently amended): The method according to claim 12, wherein the intein is selected from Saccharomyces cerevisiae See Vma intein and Mycobacterium xenopi Mxe Gyr A intein .

14. (Currently amended): The method according to claim 12, wherein the protein binding protein domain is a chitin binding domain.

Xu

U.S.S.N.: 09/786,009

Filed: April 17, 2001

Page 3

15. (Currently amended): The method according to claim 12, wherein the target protein is selected from a Bacillus stearothermophilus Bst DNA polymerase I large fragment, thioredoxin or a cytotoxic protein.

16. (Currently amended): The method according to claim 12, wherein the binding protein domain is selected from a maltose binding protein and paramyosin.

17. (Currently amended): A method for expressing a recombinant protein precursor, comprising:

(a) inserting a nucleic acid sequence encoding a target protein into a plasmid at a multiple cloning site located upstream of and in frame with a fusion gene encoding an intein and a binding protein domain, wherein

(i) the intein is selected from a naturally occurring intein, an intein derivative or an intein mutant; and

(ii) the multiple cloning site contains a linker and the linker sequence is selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4; and

(b) introducing the plasmid into a host cell and providing conditions suitable for expressing the recombinant precursor protein by the host cell.

18. (Currently amended): The method of claim 17, wherein the binding protein domain encoded by the nucleic-acid fusion gene is a chitin binding protein.

Claims 19-20 (Cancelled)

Xu

U.S.S.N.: 09/786,009

Filed: April 17, 2001

Page 4

21. (Previously added): The method according to claim 17, wherein the plasmid is a pTXB plasmid.

22. (Currently amended): A method of modifying a target protein by ligating a synthetic chemically synthesized peptide or synthetic second protein *in vitro* to the target protein, comprising:

- (a) expressing in a host cell, the target protein fused at its carboxy terminus to one of an intein selected from the group consisting of: an intein, an intein derivative or a mutant intein, the intein optionally fused to a binding protein domain at its carboxy terminus, wherein the intein is capable of thiol induced cleavage;
- (b) inducing intein mediated cleavage of the intein from the target protein by adding 2-mercaptoethanesulfonic acid so as to form a carboxy $\epsilon$ -terminal thioester on the target protein;
- (c) preparing obtaining the chemically synthesized a synthetic peptide or a synthetic second protein having an N amino-terminal cysteine; and
- (d) ligating the target protein of step (b) to the synthetic chemically synthesized synthetic peptide or a synthetic second protein of step (c) to form a modified modify the target protein.

23. (Currently amended): The method according to claim 22, wherein the protein after prior to modification is a cytotoxic protein.

24. (Cancelled)

25. (Currently amended) A method of labeling a target protein, comprising:

Xu

U.S.S.N.: 09/786,009

Filed: April 17, 2001

Page 5

- (a) expressing a recombinant precursor protein in a host cell, the precursor protein comprising the target protein fused at its carboxy terminus to an intein, the intein having an amino and a carboxy terminus such that the intein is fused at the amino terminus to the target protein and optionally fused to a binding protein domain at the carboxy terminus, the intein being selected from a naturally occurring intein, an intein derivative or an intein mutant, wherein the intein is capable of thiol induced cleavage;
- (b) cleaving the precursor protein in the presence of 2-mercaptopethanesulfonic acid so as to form the target protein having a Ccarboxy-terminal thioester;
- (c) preparing obtaining a chemically synthesized synthetic peptide or protein having a marker and an Namino-terminal cysteine; and
- (d) ligating the target protein of step (b) with the chemically synthesized synthetic peptide or protein of step (c) for labeling the target protein.

26. (Previously amended): The method according to claim 25, wherein the marker is selected from the group consisting of a fluorescent marker, a spin label, an affinity tag, and a radiolabel.

27. (Currently amended): The method according to claim 25, wherein the chemically synthesized peptide or protein fragment is an antigenic determinant.

Xu

U.S.S.N.: 09/786,009

Filed: April 17, 2001

Page 6

28. (Currently amended): A method for ligating a synthetic chemically synthesized protein or peptide to an inactive form of a protein so as to restore protein activity, comprising:

- (a) expressing in a host cell, a fusion protein comprising the first ~~target~~ inactive form of the protein fused at ~~the its~~ Ccarboxy-terminus to one of an intein, an intein derivative or an intein mutant wherein the fusion protein is expressed from a plasmid;
- (b) inducing intein mediated cleavage of the protein of step (a) by adding 2-mercaptoethanesulfonic acid so as to form a Ccarboxy-terminal thioester on the inactive protein;
- (c) preparing obtaining a chemically synthesized synthetic protein or peptide having an Namino-terminal cysteine; and
- (d) ligating the inactive form of the protein of step (b) to the chemically synthesized synthetic peptide or protein of step (c) to restore protein activity.

29. (Previously amended): The method according to claim 28, wherein the protein is a cytotoxic protein.

30. (Previously amended): The method of claim 29, wherein the cytotoxic protein is a restriction endonuclease.

Claims 31-33 (Cancelled)